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Impact of elevated CO_2 on growth, development, and reproduction of the wolf spider, *Pardosa astrigera* (Araneae: Lycosidae)

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Abstract. The effects of elevated CO_2 concentration on spiders were studied using third-instar spiderlings of the wolf spider *Pardosa astrigera* L. Koch 1878 in CO_2 climate chambers with two different concentrations of CO_2 (low, 370 ppm and high, 750 ppm). The food intake and total developmental period of spiderlings reared at high CO_2 concentration increased significantly, and the body length and weight of adult spiders decreased compared to those in the low-CO_2 group. The oviposition rate of female *P. astrigera* and the hatching rate of eggs did not differ between the high- and low-CO_2 groups, but the number of egg sacs and the total number of eggs produced by females from the high-CO_2 group decreased. These results suggest that elevated CO_2 concentrations are harmful to the growth, development, and reproduction of *P. astrigera*.

Keywords: Body length, body weight, oviposition rate

Elevated atmospheric carbon dioxide (CO_2) concentration is thought to be the main reason for global climate change and the greenhouse effect. Climatic records show that the atmospheric CO_2 concentration has increased at an annual rate of 1.9 ppm since 1995. It is estimated that the atmospheric CO_2 concentration will rise to 540–970 ppm by 2100 (IPCC 2013). Because of the increasing atmospheric CO_2 concentration, studying its effects on organisms has been a very popular subject in ecological research (Cannon 1998; Stiling & Cornelissen 2007; Ge et al. 2010; Cornelissen 2011; Foss et al. 2013). Studies to date have mostly investigated the influence of elevated CO_2 on plants, phytophagous insects, and natural enemy insects (predators and parasitoids) (Lincoln et al. 1984; Bezemer & Jones 1998; Wu et al. 2006; Chen et al. 2007; Ge et al. 2010). However, it remains unclear how an elevated CO_2 concentration impacts spiders, a key predator of insects.

Elevated atmospheric CO_2 concentrations have direct effects on the growth, chemistry, and physiology of plants (Lindroth et al. 1993; Roth & Lindroth 1995; Lin & Wang 2002; Veteli et al. 2002; Chen et al. 2005a). For phytophagous and natural enemy insects, elevated CO_2 concentration indirectly affect their growth, development, and reproduction (Brooks & Whittaker 1998; Deng et al. 2002; Chen et al. 2005b, 2007; Wu et al. 2006, 2007; Cornelissen 2011; Foss et al. 2013).

Spiders play important roles in controlling pests (Marc et al. 1999; Barrion et al. 2012) and monitoring environmental conditions (Babczynska et al. 2011; Chen et al. 2011). Understanding the effects of elevated CO_2 concentration on spiders will contribute to better protection and utilization of them. The objective of this study was to determine the effects of elevated CO_2 concentration on spiders. We investigated the food intake, molting, clutch size and egg hatch of the wolf spider *Pardosa astrigera* L. Koch 1878 under different CO_2 concentrations. Based on previous studies about predatory insects (Chen et al. 2007; Ge et al. 2010), we hypothesized that an elevated CO_2 concentration could have either harmful or beneficial effects on the spiders.

METHODS

Spider collection and rearing.—Subadult individuals of *P. astrigera* were collected from farm fields in Ma'anshan Forest Park, Wuhan (30° 52′N, 114° 31′E), Hubei Province, China, in April and May 2012. Voucher specimens were deposited in the Centre for Behavioral Ecology & Evolution, College of Life Sciences, Hubei University, China.

Spiders were kept individually in cylindrical glass tubes (diameter 2 cm, height 12 cm) with a layer of sponge (1.5 cm thick) moistened with water on the bottom. The tubes were plugged with cotton. The spiders were kept in a chamber at 24°C and relative humidity of 60–80% under a light: dark cycle of 14:10 h (lights turned on at 08:00). Every two days, we fed the spiders with adults of *Drosophila melanogaster* cultured under normal CO_2 concentration. Two days post-maturation, females and males were placed together for mating. The male was removed after the female deposited the first egg sac. Following the emergence of second-instar spiderlings and dispersal from the female's abdomen, siblings from different egg sacs were separated and reared individually, with *D. melanogaster* provided as food. Once the second-instar spiderlings molted, they were used for the following experiments.

Spiderling rearing in different CO_2 concentrations.—A total of 300 third-instar spiderlings were randomly selected from different egg sacs and reared individually in cylindrical glass tubes (diameter 2 cm, height 12 cm) and then divided into two groups (150 in each group). The spiderlings were reared under low CO_2 (370 ppm) and high CO_2 (750 ppm) concentrations in CO_2 artificial climate chambers (CC350TLHC type, Changzhou Okefenokee Instrument Co., LTD). The concentrations of CO_2 were set according to Chen et al. (2005b) and Ge et al. (2010); the current concentration of atmospheric CO_2 is 370 ppm, and 750 ppm is approximately double the current level. Each CO_2 concentration was repeated three times by using three chambers, with 50 spiderlings in each chamber. The chambers were set at 25°C and relative humidity of 40%–60% with a light: dark cycle of 14:10 h (lights turned on at

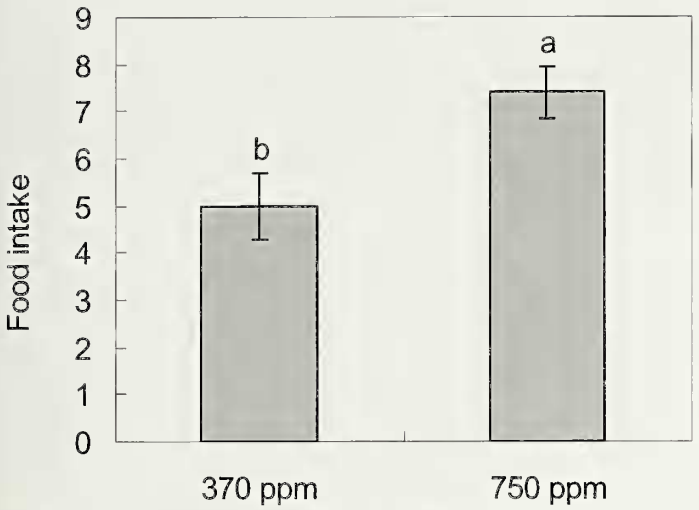

Figure 1.—Effect of different CO_2 concentrations on food intake of *Pardosa astrigera* fed on *Drosophila melanogaster* (n = 10). Different letters above the columns indicate significant differences in food intake of spiders maintained under different CO_2 concentrations (t-test, df = 9, t = 3.39, P = 0.008).

08:00). The spiderlings were fed adults of *D. melanogaster* and the sponges were replaced every two days.

Measurement of food intake of spiderlings.—The food intake of third-instar spiderlings (n = 10) under different CO_2 concentrations was investigated. Fifteen fruit flies (*D. melanogaster*) were provided at 17:00 and the number of living flies was recorded the next day at 09:00. The difference between the initial and final number of flies was taken to be the food intake of the spiderlings.

Assessment of developmental duration, body length, weight, and fecundity.—Molts were recorded when exuviae were observed in the tube, and the time between molts was used as a measure of developmental duration. Two days post-maturation, the females of *P. astrigera* (n = 15) were randomly paired with a mature male and left together for 48 h to mate. Oviposition rate, the number of egg sacs laid by the females (n = 10), the total number of eggs (n = 15), and hatching rate of the eggs (n = 4) were observed and recorded for each treatment (Chen et al. 2011). Ten female and 10 male spiders from different treatments were randomly selected and weighed to the nearest 0.01 mg by using electronic balance (FA1004N type, HANGPING). Then, the above 20 spider individuals were killed in 75% alcohol to measure the body lengths (from front edge of carapace to the end of abdomen) by using an ocular micrometer under a microscope (DFC495 type, LEICA).

Table 1.—Developmental durations (days) of spiderlings of *Pardosa astrigera* under different CO_2 concentrations. Data were expressed as Mean ± standard deviation. The differences between different CO_2 concentrations were compared by Student's t-test. The same notation is used in Tables 2– 4.

Items	CO_2 concentration (ppm) 370	750	t	P
3rd instar	15.4 ± 0.6	20.6 ± 0.9	2.31	0.025
4th instar	13.2 ± 0.5	19.0 ± 0.7	2.29	0.031
5th instar	16.3 ± 0.9	25.1 ± 0.4	2.97	0.008
6th instar	17.1 ± 0.9	22.4 ± 0.4	2.58	0.022
Total	61.8 ± 2.4	87.1 ± 1.5	2.96	0.0037

Table 2.—Body lengths (mm) of adult *Pardosa astrigera* under different CO_2 concentrations (n = 10).

Sex of spider	CO_2 concentration (ppm) 370	750	t	P
Female	9.70 ± 0.84	7.10 ± 0.42	2.66	0.026
Male	8.30 ± 0.45	5.40 ± 0.42	3.14	0.012

Statistical analysis.—Data were expressed as mean ± standard deviation. The differences between treatments were compared by Student's t-test.

RESULTS

The consumption of *D. melanogaster* by the third-instar spiderlings of *P. astrigera* reared at the high CO_2 concentration was significantly higher than at the low CO_2 concentration (t-test, df = 9, t = 3.39, P = 0.008) (Figure 1).

The duration of development (days) for each instar (t-test, $P < 0.05$) and the total duration (t-test, $df1$ = 65, $df2$ = 53, t = 2.96, P = 0.0037) for the spiderlings were significantly longer for the spiderlings kept under high CO_2 concentration than under low CO_2 concentration (Table 1). This suggested that elevated CO_2 caused the spiderlings to mature later, prolonging the total development time.

The body length of female *P. astrigera* was longer than in males kept under the same CO_2 concentration (Table 2). Body lengths of both adult females (t-test, df = 9, t = 2.66, P = 0.026) and males (t-test, df = 9, t = 3.14, P = 0.012) of the high-CO_2 group were shorter than those of the low-CO_2 group. This showed that the body lengths of mature spiders were decreased under the high CO_2 concentration.

The body weight of females of *P. astrigera* was also higher than that of males under the same CO_2 concentration. The body weights of both female (t-test, df = 9, t = 4.78, P = 0.001) and male spiders (t-test, df = 9, t = 2.92, P = 0.017) reared at the low CO_2 concentration were heavier than those reared at high CO_2 (Table 3).

There were no differences in the oviposition rate of female *P. astrigera* (t-test, df = 14, t = 0, P = 1.0) or the hatching rate of eggs (t-test, df = 3, t = 1.01, P = 0.37) between the high- and low-CO_2 groups (Table 4). However, the number of egg sacs produced by the females from the high-CO_2 group was lower than the number produced by the low-CO_2 group (t-test, df = 9, t = 2.69, P = 0.025), and the total number of eggs of the high-CO_2 group was also lower than in the low-CO_2 group (t-test, df = 14, t = 3.49, P = 0.0036).

Table 3.—Body masses (mg) of adult *Pardosa astrigera* under different CO_2 concentrations (n = 10).

Sex of spider	CO_2 concentration (ppm) 370	750	t	P
Female	41.3 ± 8.6	33.5 ± 2.7	4.78	0.001
Male	31.9 ± 0.6	22.4 ± 0.5	2.92	0.017

Table 4.—Fecundity of female *Pardosa astrigera* under different CO_2 concentrations

Items	CO_2 concentration (ppm) 370	750	t	P
Oviposition rate (%)	100 ± 0	100 ± 0	0	1.0
Number of egg sacs	5.6 ± 0.9	2.8 ± 0.8	2.69	0.025
Total number of eggs	229.6 ± 39.8	125.3 ± 34.9	3.49	0.0036
Hatching rate (%)	100 ± 0	83.7 ± 15.6	1.01	0.37

DISCUSSION

Under a high concentration of atmospheric CO_2, the nitrogen content of plants decreases, and defoliating insects will increase their feeding rate in order to compensate for the nutritional loss caused by the lower nitrogen content of the host plant (Lincoln et al. 1984). Chen et al. (2007) found that the larvae of lady beetles (*Harmonia axyridis*) consumed more cotton aphids *Aphis gossypii* fed on cotton plants grown in elevated CO_2 in order to compensate for the reduced soluble protein in *A. gossypii* owing to the decrease in foliar N and the increase in the C:N ratio in the cotton plants. Our results showed that the third-instar spiderlings of *P. astrigera* also consumed more fruit flies at the high than at the low CO_2 concentration. This phenomenon may have been caused by the larger amounts of energy required by the spiders and higher respiration rates (Foss et al. 2013) to compensate for their increased activity in the high-CO_2 environment.

High atmospheric CO_2 concentration could affect enzyme activity in the cotton bollworm *Helicoverpa armigera*, and the available nutrients would decline significantly, thereby impairing the growth and development of *H. armigera* (Chen et al. 2005b). The body length and weight similarly decreased in *H. armigera* (Chen et al. 2005b). When second-stadium gypsy moth (*Lymantria dispar*) larvae were grown under high CO_2 concentration, their body weight was lower than in the normal CO_2 concentration (Wang et al. 2006). Our former results showed that when *P. astrigera* was subjected to the stress of Pb or Zn, its developmental duration lengthened (Chen et al. 2011). Similar results were found in our present study. The development time of *P. astrigera* lengthened and the body length and weight of mature spiders decreased. This suggests that these different environmental factors can cause developmental delay in this spider species.

Our results indicated that the fecundity of *P. astrigera* was significantly decreased at the high CO_2 concentration. Brooks & Whittaker (1998) found that the number of eggs produced by females of the green dock beetle *Gastrophysa viridula* that were continuously cultured for three generations, decreased under a high atmospheric CO_2 concentration. At the high atmospheric CO_2 concentration, the number of eggs laid by females of *H. armigera* was markedly reduced (Chen et al. 2005b). However, some studies have had different outcomes. Bezemer & Jones (1998) showed that the number of eggs laid by females of the winter moth *Operophtera brumata* could increase, and the number of eggs laid by *G. viridula* did not differ significantly between high and normal CO_2 concentrations (Brooks & Whittaker 1998). In the present study, female spiders laid fewer eggs under the stress of high CO_2 concentration. Taken together, these studies show that the impact of elevated CO_2 on the reproduction of arthropods varies among species.

In summary, the high CO_2 concentration proved to be harmful to the growth, development, and reproduction of *P. astrigera*. The results of this experiment increase understanding of the responses of spiders reared in elevated atmospheric CO_2 concentration.

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